AD NUMBER

AD838930

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; JAN 1964. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

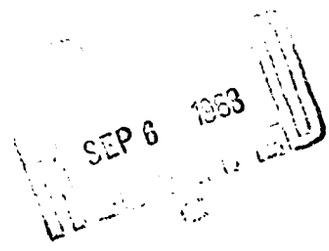
AUTHORITY

SMUFD D/A ltr, 14 Feb 1972

TRANSLATION NO. 1328

~~DATE: 63~~

DATE: JAN. 4, 1964



B

1328

The Medical Press 72. No. 1, Jan. 4, 1964
Pages 21-24

Biological Movement

The Notion of Interferon in Virology

By (Mrs.) Y . Perol

A cell parasited by a virus acquires a resistance to infection by another virus, be it of the same group or of a different group. This cellular response seems to have been observed by Jenner in 1805 when he notes that the vaccinal lesion appeared with less regularity when herpism is present.

Findlay and Mac Callum in 1937 show that monkeys infected by the Rift Valley Fever virus are protected against yellow fever and name this phenomenon "viral interference"; actually, it is not, in this case, a question of an immunitary phenomenon due to some antibodies; these two viruses do not have any antigenic relationship and cannot provoke crossed immunity. The same year, Dalldorf, Douglas and Robinson report that the monkeys infected with the virus of the chorio-meningitis lymphocyte do not become paralyzed when they are super-infected by poliomyelitis viruses. This protection lasts two weeks, then diminishes and, after a month, the monkeys find again their entire susceptibility to the poliomyelitis. More than that, the spinal marrows of the monkeys doubly infected contain less of the poliomyelitis virus than the spinal marrows of the control monkeys, which suggests an inhibition of the viral multiplication.

In 1943, Werner and Henie show that a virus inactivated even by heat or ultra-violet rays can induce the interference.

It is finally in 1957 that Isaacs and Linderman discover the interferon.

Discovery and Definition of the Interferon

Inocculating a grippal virus inactivated by the heat in chorio-allantoidian (M.C.A.) membrane of the egg of an incubated chicken, these authors state, a few hours later, that the M. C. A. washed and secondarily infected with alive grippal virus, no longer permits the multiplication of the virus. Far

more, the liquid soaking the cells but deprived of them, deposed on the new cells, render them resistant to the infection by a gamut of varied viruses. The substance responsible for the interference phenomenon is extract and named interferon.

It is a cellular protein:

--- snythesized by the host cell, in the course of viral infection:

---present in the infected cell;

---soluble, diffusing in the medium.

It is not neutralized by the viral antibodies:

It is thus absolutely different from a viral antigene;

In no way is it a part of the viral particle.

The infected cell synthesizes virus and interferon at the same time. Finally, it is not a question of a property acquired definitely by the cell, but of a reversible phenomenon:

---it disappears with the infection;

---it exhausts itself after cellular multiplication due to the fact of the dilution.

Production of Interferons in Tissue Culture

In vitro a tissue culture parasited by a virus is equally a producer of interferon.

Human kidney cells or human amnios infected with a stock of apathogenic polio virus II adapted to the chicken embryo, produce a lot of interferon. Three to four days after the

A cell parasited by a virus acquires a resistance to the infection by all other viruses. This non-specific resistance rests on the production of a protein substance, interferon. This substance diffuses in the culture mediums and it has been shown that it concerns a protein substance molecule of very inferior dimensions to those of the virus and of the antibodies. It would exercise its action in the midst of the cell by blocking the synthesis of the viral nucleic acid. This discovery furnished the explanation of the numerous virologic problems. The therapeutic applications, up until the present very limited, are susceptible to an extension actually unforeseen.

infection, the medium surviving on the tissue culture, that presents no injury, possesses an interferent activity: collected and deposited on the new tissue cultures, it protects them against the activity of a polio virus I or of a Sindbis virus, from the vaccine of an Echo 9 (Ho and Enders).

I. Numerous virus cell systems are thus producers of interferal substances:

I° Cells of the first explantation secrete interferon:

the fibroblasts of the embryo of mice infected by Newcastle's virus;

the cells of the embryo of the chicken infected by the Sindbis virus.

2° Cells in a continuous line, like the Hela cell infected by a poliomyelitic virus attenuated (Ho and Enders), or the Kb cell infected by Myxovirus parainfluenzae III (Chany), or the L cell infected by the virus of the encephalic equine of the West, produce interferon in their culture medium.

3° In cellular cultures chronically infected, called "carrying cultures", of interferon was put as evidence in the surviving medium. Of such cultures: human cells chronically infected by Newcastle's virus, these are resistant to the infection by the virus of the vesiculose stomata. The secreted interferon seems responsable for the prolonged chronic infection due to the fact of an equilibrium between the degree of viral infection and the production of the inhibitor(9).

II. ---the inductor viruses. Nearly all of the viruses can induce the production of interferon: the arbor viruses and the myxo-viruses (such as the influenza virus or the measles virus) seem particularly active, but also the entero viruses, the pox viruses, the oncogenic viruses (polyome, the sarcoma of Rous virus).

It is worthy to notice that, among these viruses, there are some of RNA, there are some of DNA; there are some of very different dimensions, that which suggests that the capacity to induce the interferon would be a general property of the viruses which parasite a cell. The myxo viruses, even when inactivated by the ultraviolet rays or the heat, keep their inducing power on the condition that one sollicits the cell with a massive dose of inactivated virus. The virus inactivated by the ultraviolet rays remains a better inductor than the heated virus, and the incomplete particles of the influenza virus can induce interference. But it seems that an inactivated virus is only a good inductor for a cell when the reproductive power of the infectious virus in the studied cell is removed (18).

III. ---Nucleic acids can equally be inductors. One knows that viral nucleic acids, that is to say, the virus stripped of its protein and reduced to its nucleic acid (support of its genetic material), can induce infection and equally the production of interferon. Even more, it seems that non-viral nucleic acids, being the source of foreign cells, can induce the formation of an interferon (Isaacs).

IV. ---Kinetics of the production of interferon. With an inactivated virus inoculated with massive doses, interferon appears rapidly and its production is maximal in a few hours, whereas with an infective virus, the interferon appears in a parallel manner to the production of daughter viral

particles and only attains its maximum after the infectious concentration has attained its maximum.

V. ---A temperature of 37° is necessary to the production of interferon by the cells. At 30°, while the viral multiplication is only inhibited, there is no longer any production of interferon.

How is Interferon Prepared?

Whether it is a question of the allantoic liquid of the egg of an infected chicken with some influenza virus or of the medium surviving a tissue culture infected by an inductive virus, the liquids are gathered up and all of the viral particles are eliminated by ultracentrifugation or dialysis in the acid medium, then eventually blocked by an antiviral serum corresponding in excess. The obtained solution is neutralized: it represents the crude interferon that can be conserved at a cold temperature at +4° for several months.

Physical Properties of Interferons

The interferon contained in these solutions is a protein and as such:

---it is destroyed by pepsin or trypsin;

---it does not contain nucleic acid as it is not inactivated by digestion by the desoxyribonucleus or the ribonucleus;

---it is stable in an acid medium (of 2 pH to 10 pH), which explains the dialysis in the acid medium in order to separate it from all viral particules, few of the virus resisting twenty-four hours at pH 2;

---it is instable to the heat, destroyed by heating for one hour at 60°C, resistant to ether.

it is a molecule of very little size: the ultracentrifugation which precipites the smallest viruses, allows the interferent activity to persist in the supernatant.

Purification of Interferon

It is attempted by diverse procedures. Lampson and Hilleman (15), after precipitation by zinc acetate, chromatography on column and electrophorus of zone on pevikon, obtain a purified interferon that is a basic protein at the isoelectric point bordering on 8, of small molecular weight, approximately 20 000 to 34 000, very sensitive to the action of ultra-violet rays.

More recently Rotem and Charlwood (21), by sedimentation in drops of density, demonstrated that the interferons produced by the embryo cells of chicken, of mice embryos, of monkey kidneys in tissue culture, have the same coefficient of sedimentation 1,9 s, which replies to a molecular weight comprised between 13 000 and 20 000 to 25 000.

Test of the Activity of the Interferon "IN VITRO"

The principal of it is the folliwing:

In a first time:
One makes the interferon solution act at progressive dilutions on the tissue cultures; one leaves it to incubate twenty-four hours so that its protection can establish itself.

In a second time:
One provokes the cultures with a virus of which one appreciates parallely the infectious power on the test cultures stripped of interferon, be it that it multiplies itself there in giving an elevated standard, be it that it creates visible cellular lesions. One juges the degree of protection due to the interferon on the inhibition of the viral multiplication or on the inhibition of the cellular lesions.

The most utilized cell-virus pair is the Sindbis virus and the human amniotic cells.
For example: Cultures of amniotic cells are treated twenty-four hours by growing dilutions of the interferon solution. Then to each culture one adds 100 DL 50 of Sindbis virus, as well as to the test cultures.
The following days, one inspects the integrity of the culture: a good solution of interferon inhibits up to 1/64.
or again: one leaves the tubes of tissue culture, treated by dilutions of interferon during twenty-four hours, then inoculated with the revealing virus.
A few days later, one tests parallely the virus in the test culture tubes, and in the culture tubes treated with interferon.
Most of the testing techniques are based on the method of the region of lysis provoked by a virus on tissue culture on Petri dish, the unity of interference expressing itself by the dilution that reduces by 50% the number of regions.

Biological Properties of Interferons: Their Action on the Cells

Many works have established the following points:

1. Notion of the sensible cell. In the laboratory the cells of first explantation, human amnios for example, are good producers of interferon and are sensible to its action; incubated in its presence, they become resistant to the viral infection.
The neoplasic human cells in a continuous line (Hela or Kb), equally producers of interferon, are hardly sensible to its action and cannot be utilized to test them: it seems that the metabolism of the cancerous cells does not permit the interferon to exercise its anti-viral action on the cell.
The cells of the chicken embryo (Isaacs and Baron) behave differently according to their age: before the seventh day,

even if their metabolism is around that of the newly grown cells, they cannot fabricate interferon and are insensible; it is only around the tenth day that they can produce interferon and become sensible to its action.

2. Cellular Specificity of interferons. In most cases, the protective action of an interferon is connected to the cellular species that produced it: it is thus that an interferon secreted by calf cells, protects much better the calf cells against a viral infection than the chicken cells. This cellular specificity is not absolute, the interferon prepared from the kidney cells of a monkey protects in vitro cells of human origin.

3. Metamorphogenic Action of certain interferons. The interferon induced on amniotic human cells by the Sendai virus, provokes at a sufficient concentration a morphological modification of amniotic cell cultures whose hexagonals become spindle-shaped, of fibroblastic allure (Gresser). This phenomenon is reversible.

4. Preliminary Incubation. A solution of interferon deposited on a sensible new tissue culture does not confer upon it the maximum resistance to the infection except after the preliminary incubation of several hours; however, at an elevated concentration, it could have had an inhibiting effect even when it had been given after the beginning of the infection.

5. The protective effect of the interferon does not follow the law of all or nothing (14). This effect goes from the suppression of the cellular infection to a partial inhibition of the viral synthesis. But the degree of protection does not seem directly proportional to the dose of interferon. Perhaps it is necessary to invoke a heterogeneous cellular receptivity, a small number of resistant cells and producers of interferon sufficing to protect the others.(7)

6. The duration of the protection that the interferon confers to the cells is variable, going from a few hours to a few days; it persists for a longer time in the absence of cellular division. But if the cells divide themselves actively, the concentration of interferon lowers rapidly in each cell and the cells become sensible again to a new viral infection.

7. The inhibited viruses. The protection conferred to the cell by the interferon exercises itself toward numerous viruses: arbor, myxo-virus, vaccine, enterovirus, adenovirus, tumoral virus and even towards herpetic virus(9). And this whatever be the virus that induced the production of interferon.

8. Action mecanism. Despite all these experimental data, the action mecanism of the interferon rests unknown for

the moment.

One knows that it acts on the cell and not on the virus; the interferon does not inactivate the extracellular virus not the extracellular infectious viral ribonucleic acid either.

When a virus parasites a cell, the cycle of events unrolls in the following order:

1. Absorption of the virus on the cellular wall;
2. Penetration of viral nucleic acid in the cell;
3. Eclipse phase, during which the virus is no longer detectable;
4. Apparition of the nucleic acid of the daughter virus;
5. Synthesis of the viral protein;
6. Liberation of complete viral particles.

The cells protected by interferon absorb the virus like normally susceptible cells. Likewise, the virus penetrates normally the cell, but the viral multiplication is blocked.

The interferon exercises its inhibiting action toward viruses very different as to their size or their structure; the cellular protection is effective even when one provokes the cell by an infectious viral ribonucleic acid. It thus seems that the interferon acts on a key phase of the viral multiplication.

It was shown(8) that this action situates itself during the eclipse phase: it seems to block the synthesis of the viral carrier ribonucleic acid.

But by which mecanism?

Isaacs showed that the interferon provokes in the cells a weak augmentation of the oxygen consumption and an augmentation of the oxygen consumption and an augmentation of glycolysis; but the hypothesis according to which the interferon would act in breaking the joinings of couplings of reactions of oyxdative phosphorylation seems abandonned today, as the interferon is active as well in aerobiosis as in anaerobiosis (Zemla).

Production of Interferon "IN VIVO"

If it is relatively easy to produce interferon in infected tissue culture and put in evidence its protective action on a tissue culture, it is much more difficult

1. to demonstrate its presence in the living being infected by a virus,

2. to prove that it plays a role in the cure of the viral infection.

Hitchcock and Isaacs, inocculating mice by the intranasal method with influenza viruses, demonstrated the production of interferon in pulmonary tissue.

They established in a parallel manner:

__ the curve of viral multiplication in the pulmonary tissue: it presents a maximum on the third day;

__ the appearance of interferon of which the maximum production situates itself from the third to the fifth day, and then declines;

__ the ascension of the serous antibodies, much more tardy, beginning from the second week.

In the course of this experience, the animal is protected against encephalite due to infection by the Bunyamvera, the multiplication of the virus being inhibited. The same protection is obtained by the intraperitoneal injection of an interferon prepared on tissue culture.

In this way, the production of interferon is demonstrated in situ in response to viral infection. If it is certain that the healing of the animal is not due to the production of antibodies too tardy, it is demonstrated that the production of interferon by the infected cells plays a role in this healing. The phenomenons of interference observed in vivo in the animal with the viruses whose election cells are situated in points removed from the organism: the influenza virus and the hemorragic encephalitic virus (23) suggested the hypothesis that interferon produced in situ diffuses by sanguine method to the sensible cells of the organism.

The human white globules, of which one knows that they can fix many viruses and beginning from which they recently isolated the measles virus, on the first day of the morbiliform eruption (11), produce interferon in great quantity when they are maintained in survival and sollicited by the measles virus (Gresser). Is it of the same nature in the sanguine current? It is not demonstrated.

In the mouse, the white blood corpuscles of a peritoneal exsudate sollicited by an inactivated vaccinal virus, fabricate interferon. Transferred by intraperitoneal method to new mice, it protects them against the intracerebral inocculation of the vesiculose stomatitis virus (24).

Practical Applications

It is thus certain that interferon is a natural method of immediate defense in viral sicknesses.

1. Its therapeutic action, proved in vitro in the laboratory on "supporting tissue cultures" chronically infected by the vaccine or herpes (9), of which one was able to obtain the recovery, was confirmed in vivo on the animal(10).

Interferon injected by intradermic method, twenty-four hours previously, protects in situ the rabbit's skin against the vaccine (Lindenman, Isaacs, Westwood); injected by parenteral method, it protects the mouse against Bunyamvera virus (Hitchcock), against the West Nile virus (Vainio and coll.), the chicken embryo against the equine encephalitic virus of the

West (Wagner).

An interferon prepared on malignant cells of hamster protect the newly born hamster against the tumors induced by the polyome virus.

It seems that in slowing up the multiplication of this oncogenic virus, the animal can make antipolyome antibodies and acquire a resistance that it keeps until adult age (Atanasiu and Chany).

In man, a few therapeutic attempts were tried with an interferon produced on a culture of monkey renal cells. A rapid cicatrization of epithelial ulcerative lesions of vaccinal keratosis by ocular instillations of interferon was obtained, as well as the suppression of the local inflammatory reaction of the antivariolic vaccination after intradermic injection of interferon twenty-four hours previously.

These discreet efforts, all of them arising from a local treatment where the interferon is put in contact with the cells to protect, practiced with a very insufficiently purified interferon, brings us however to pose the problems raised by an eventual therapeutic utilization of interferons.

If up until the present the preparations of interferon are revealed stripped of toxicity and stripped of antigenicity, it would be surprising that this foreign protein should absolutely not be antigenic. Recently Paucker and Cantell, beginning from an interferon produced by mice cells, were able to prepare on the guinea pig an anti-interferon serum neutralizing its activity (19). Finally, and above all, as all viral therapeutic, even if it is efficacious and inoffensive, it strongly risks to be administered in the course of viral infection acquired at a stage where it can only at the very most limit cellular lesions already definitive.

2. The discovery of interferon enlightens greatly pathogenic problems or viral epidemiologic problems. The injurious action of corticotheraphy in certain viral sicknesses can perhaps be explained by the fact that cortison inhibits the production and the action of the interferon (Kilbourne, Smart and Pokorny).

In the course of oral vaccinations by a living antipoliomyelitic vaccine in Mexico, many children do not fabricate antibodies; they were lodging an enterovirus at the epoque in which they received the vaccine, and this enterovirus, acting by interference, prevented the development of the attenuated antigenic living virus. Also, the vaccinations by living oral vaccine are advised against in summer, the season where enterovirus infections predominate.

3. In the culture tissue techniques, applied to the diagnosis of viral sicknesses, it is necessary from now on to take into account interferences.

The tissue cultures can be parasited by latent viruses (20), liberating interferon in the culture medium, and hindering isolation after the scattering of the suspect organic product.

On the other hand, the presence of the German measles virus isolated on the cultures of the monkey kidney was only disclosed thanks to the resistance of these cultures to an ECHO II virus (Buescher and Parkman) (22), resistance transmissible by passage in serie.

In summation, interferon is a protein distinctly smaller than a virus and an antibody, produced by animal cells of different kinds, under the action of most of the living viruses or inactivated viruses, inhibiting most of the viruses by blocking the intracellular synthesis of viral nucleic acid in cells of the same kind (but this cellular specificity is not absolute), this to doses that are not toxic for the cells.

The discovery of interferon enlightens by a new day the cellular resistance acquired in viral infection. However, if the tissular immunity is definitive, the protection by interferon is only temporary and ceases with the infection. There exists in man chronic viral infections: one knows the frequence of adenoviruses in human tonsils, it is possible that they play a protective role.

Bibliography

1. Atanasiu, P. : Interferon, the first applications in vivo. Medecine et Hygiene, March 14, 1962, 20, 208-209.

2. Garell, C. Dale: Resistance to viral infection. American Journal of Diseases of Children, January 1963, vol. 105, 106-113.

3. Golde, A. : Interferons. Revue francaise d'etudes cliniques et biologicues,(French Review of clinical and biological studies), 1963, 8, 80-87.

4. Isaacs, A. : Interferon. Scientific American, May 1961, vol. 204, # 5, 51-57.

5. H o, M. : Role of infection in viral interference. Archives of International Medicine, November 1962, vol. 110, 653-659.

6. Schlesinger, R. W. : Interference in viral and rickettsial infections of Man. Rivers and Horsfall, 145-156.

7. Wagner: Cellular resistance to viral infection, with particular reference to endogenous interferon. Bacteriological Reviews, March 1963, vol. 27, #1, 72.

8. De Sommer, P., Prinzie, A ., Denys, P. and Schonne, E. : Mechanism of action of interferon. Relationship with viral ribonucleicacid. Virology. January 1962, 16, 63-70.

9. Glascow, L. A. and H abel, K. : Interferon production by mouse leukocytes in vitro and in vivo. The Journal of

Experimental Medicine, January 1963, 117, 149-160.

11. Gresser, I. and Chany, C. : Isolation of measles virus from the washed leucocytic fraction of blood. P. S. E.-B. M., July 1963, 113, 695-698.

12. Grossberg, S., Hook, E. and Wagner, R. : Hemorrhagic encephalopathy in chicken embryon infected with influenza virus. III. Viral interference at a distant site induced by priorallantoic infection. Journal of Immunology, 1962, 88, 168.

13. Grossberg, S. E. and Holland, J. J. : Interferon and viral ribonucleic acid. Effect on virus susceptible and insusceptible cells. Journal of Immunology, June 1962, 88, 708-714.

14. Ho, M. : Kinetic consideration of the inhibitory action of an interferon produced in chick cultures infected with Sindbis virus. Virology, June 1962, 17, 262-275.

15. Lampson, G. P., Tytell, A. A., Nemes, M. M. and Hilleman, M. R. : Purification and characterization of chick embryon interferon. P. S. E. B. M., 1963, 112, 468-478.

16. Lindenmann, J. and Gifford, G. E. : Studies on vaccinia virus plaque formation and its inhibition by interferon. Virology, 1963, 19, 284-293, 294-301, 302-309.

17. Lockart, R. Z. and Horn, B. : Interaction of an interferon with L cells. Journal of Bacteriology, May 1963, 85, 996-1002.

18. Paucker, K., Skurska, Z. and Henle, W. : Quantitative studies on viral interference in suspended L cells. Virology, 1962, 17, 301-311, 312-323, 324-334.

19. Paucker, K. and Cantell, K. : Neutralization of interferon by specific antibody. Virology, 1962, 18, 145-146.

20. Plummer, G. : Interfering properties of Sumian viruses. British J. of Experimental Pathology, February 1963, 44, #1, 58-65.

21. Rotem, Z. and Charlwood, P. A . : Molecular weights of interferons from different animal species. Nature, 1963, 198, # 4885, 1066-1068.

22. Veronelli, J. A., Maassab, F. and Hennessy, A. V. : Isolation in tissue culture of an interfering agent from patients with Rubella. P. S. E. B. M., 1962, 111, 472-476.

23. Wagner, R. R. : Biological studies of interferon? II. Temporal relationships of virus and interferon production by cells infected with eastern equine encephalomyelitis and influenza viruses. Virology, 1963 19, 215-224.